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<b>(21) International Application Number:</b> PCT/US96/08629 <b>(22) International Filing Date:</b> 4 June 1996 (04.06.96) <b>(30) Priority Data:</b> 08/463,911 5 June 1995 (05.06.95) US <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 463,911 (CIP) Filed on 5 June 1995 (05.06.95) <b>(71) Applicant (for all designated States except US):</b> WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH [US/US]; Nine Cambridge Center, Cambridge, MA 02142 (US) <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SCHERER, Philipp, E. [CH/US]; 316 Lexington Street, Watertown, MA 02172 (US). LODISH, Harvey, F. [US/US]; 195 Fisher Avenue, Brookline, MA 02146 (US). <b>(74) Agents:</b> GRANAHAN, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).		<b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> SERUM PROTEIN PRODUCED EXCLUSIVELY IN ADIPOCYTES		
<b>(57) Abstract</b> <p>The present invention relates to DNA encoding Acrp30, of vertebrate (e.g. mammalian) origin, and particularly of human and rodent origin. The present invention further relates to isolated, recombinantly produced or synthetic DNA which hybridizes to the nucleotide sequences described herein and RNA transcribed from the nucleotides sequence described herein. In addition, the invention relates to expression vectors comprising DNA encoding Acrp30, which is expressed when the vector is present in an appropriate host cell. The invention further relates to isolated, recombinantly produced or synthetic mammalian Acrp30 of vertebrate (e.g., mammalian) origin, and particularly of human and rodent origin. Also encompassed by the present invention is an inhibitor or enhancer of Acrp30. The present invention further relates to a method of identifying inhibitors or enhancers of Acrp30. Isolation of Acrp30 makes it possible to detect Acrp30 or adipocytes in a sample (e.g., test sample). In addition, the present invention relates to a method of regulating the energy balance (e.g., nutritional status) of a mammal by administering to the mammal an inhibitor or enhancer of the Acrp30. The present invention further relates to a method of modulating insulin production in a mammal comprising administering Acrp30 to the mammal.</p>		

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## SERUM PROTEIN PRODUCED EXCLUSIVELY IN ADIPOCYTES

RELATED APPLICATIONS

This application is a Continuation-in-Part of  
copening U.S. Patent Application Serial No. 08/463,911,  
5 filed June 5, 1995, entitled "A Novel Serum Protein  
Produced Exclusively In Adipocytes", by Philipp E. Scherer  
and Harvey F. Lodish, the entire teachings of which are  
incorporated herein by reference.

BACKGROUND OF THE INVENTION

10 Fat cells or adipocytes are a principal storage depot  
for triglycerides, and are thought to be endocrine cells.  
Adipocytes are the only cell type known to secrete the *ob*  
gene product and adipsin, which is equivalent to Factor D  
of the alternative complement pathway (Zhang, Y., et al.,  
15 *Nature* 425-432 (1994); Spiegelman, B.M., et al., *J. Biol.*  
*Chem.* 258:10083-9 (1983)). The *ob* gene product is believed  
to be involved in the signalling pathway from adipose  
tissue that acts to regulate the size of the body fat  
depot. Mice homozygous for a defect in the *ob* gene become  
20 morbidly obese (for a review see Rink, T., *Nature*,  
372:(1994)). However, little else is known about fat  
storage mechanisms or energy balance regulation.

A greater understanding of genes involved in  
regulating fat storage in an organism will provide new  
25 approaches for the treatment of a variety of conditions  
involving the energy balance and/or nutritional status of a  
host, such as obesity, obesity related disorders and  
anorexia.

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SUMMARY OF THE INVENTION

The present invention is based on the discovery and isolation of a gene encoding a 30 kD protein produced exclusively in adipocytes. As shown herein, the protein, which is designated adipocyte complement related protein (Acrp30), is secreted by adipocytes; insulin alters (inhibits or enhances) secretion of Acrp30 from adipocytes. Evidence provided herein indicates that Acrp30 is involved in the energy balance (e.g., the nutritional status) of a vertebrate (e.g., a mammal).

The present invention relates to DNA encoding Acrp30, of vertebrate (e.g., mammalian) origin, and particularly of human and rodent origin. The DNA of the present invention can be isolated or purified from sources in which it occurs in nature, recombinantly produced or chemically synthesized. The DNA of the present invention includes DNA encoding murine Acrp30 (SEQ ID NO:1), DNA encoding human Acrp30 (SEQ ID NO:6), DNA encoding other vertebrate Acrp30 and portions thereof which either encode vertebrate Acrp30 or which are characteristic of Acrp30-encoding DNA and can be used to identify nucleotide sequences which encode Acrp30 (e.g., a nucleic acid probe), as well as to complements of the forgoing sequences.

The present invention further relates to isolated, recombinantly produced or synthetic DNA which hybridizes to the nucleotide sequences described herein and encodes Acrp30 (i.e., a protein having the same amino acid sequence) or encodes a protein with the same characteristics of Acrp30. In particular, the invention relates to DNA which hybridizes to SEQ ID No: 1, SEQ ID No: 6, other sequences which encode vertebrate Acrp30 or portions thereof. RNA transcribed from DNA having the nucleotide sequence of SEQ ID No: 1, a complementary sequence of SEQ ID NO:1, SEQ ID No: 6, a complementary sequence of SEQ ID NO:6, DNA encoding other vertebrate

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Acrp30 or portions thereof are also encompassed by the present invention.

In addition, the invention relates to expression vectors comprising DNA encoding Acrp30, which is expressed when the vector is present in an appropriate host cell. In particular, the expression vector of the present invention comprises the nucleotide sequence of SEQ ID No: 1, SEQ ID No: 6 or portions thereof.

The invention further relates to isolated, recombinantly produced or synthetic Acrp30 protein of vertebrate (e.g., mammalian) origin, and particularly of human and rodent origin. The Acrp30 of the present invention has the amino acid sequence of SEQ ID No: 2, the amino acid sequence of SEQ ID No: 7, an amino acid sequence of other vertebrate Acrp30, or portions thereof which have the same characteristics as Acrp30 as described herein.

Also encompassed by the present invention is an agent which interacts with Acrp30, directly or indirectly, and alters (inhibits or enhances) Acrp30 function. In one embodiment, the agent is an inhibitor or agonist which interferes with Acrp30 directly (e.g., by binding Acrp30) or indirectly (e.g., by blocking the ability of Acrp30 to interact with or bind a molecule which it normally interacts with or binds in order to function). In a particular embodiment, the inhibitor is an antibody specific for Acrp30 or a portion of Acrp30 protein; that is, the antibody binds the Acrp30 protein. For example, the antibody can be specific for the protein encoded by the amino acid sequence of rodent Acrp30 (SEQ ID No: 2), the amino acid sequence of human Acrp30 (SEQ ID No: 7) or portions thereof. Alternatively, the inhibitor can be an agent other than an antibody (e.g., small organic molecule, protein, peptide) which binds Acrp30 and blocks its activity. For example, the inhibitor can be an agent which mimics Acrp30 structurally but lacks its function.

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Alternatively, it can be an agent which binds or interacts with a molecule which Acrp30 normally binds or interacts with, thus blocking Acrp30 from doing so and preventing it from exerting the effects it would normally exert. In another embodiment, the agent is an enhancer of Acrp30 which increases the activity of Acrp30 (increases the effect of a given amount or level of Acrp30), increases the length of time it is effective (by preventing its degradation or otherwise prolonging the time during which it is active) or both, either directly or indirectly.

The present invention further relates to a method of identifying inhibitors or enhancers of Acrp30. An inhibitor of Acrp30 interferes with the function or bioactivity of Acrp30, directly or indirectly. An enhancer of Acrp30 enhances the function or bioactivity of Acrp30, also directly or indirectly.

Isolation of Acrp30 makes it possible to detect Acrp30 or adipocytes in a sample (e.g., test sample). In one embodiment, Acrp30 encoding DNA or RNA is detected. In this embodiment, the sample is treated to render nucleic acids in cells in the sample available for hybridization to a nucleic acid probe. In one embodiment, the nucleic acids in the sample are combined with a nucleic acid probe (e.g., labeled) comprising all or a portion of the nucleotide sequence of mammalian Acrp30, under conditions appropriate for hybridization of complementary nucleic acid sequences to occur. For example, the nucleic acid probe comprises the nucleotide sequence of SEQ ID No: 1, the complement of SEQ ID NO:1, SEQ ID No: 6, the complement of SEQ ID NO:6, or portions thereof. Specific hybridization of a sequence in the treated sample with the nucleic acid probe indicates the presence of nucleic acid (DNA, RNA) encoding mammalian Acrp30. In a second embodiment, Acrp30 protein is detected. In this embodiment, the sample is combined with an antibody directed against all or a portion of mammalian

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Acrp30 and specific binding of the antibody to protein in the sample is detected. The occurrence of specific binding of the antibody indicates the presence of Acrp30 in the sample. An antibody directed against Acrp30 can also be used to detect the presence of adipocytes in a sample, such as in cultured cells such as primary or secondary (non-immortalized cells) cells or cell lines.

In addition, the present invention relates to a method of regulating the energy balance (e.g., nutritional status) of a mammal, by administering to the mammal an agent (e.g., an inhibitor or an enhancer of the Acrp30) which interacts with Acrp30, either directly or indirectly. This method can be used to decrease weight gain in a mammal (e.g., for conditions related to obesity) or conversely, to increase weight gain in a mammal (e.g., for conditions related to anorexia).

The present invention further relates to a method of modulating (enhancing or inhibiting) insulin production in a mammal (e.g., human) comprising administering Acrp30 to the individual (e.g., using cells which contain DNA which encodes Acrp30 which is expressed and secreted).

The data presented herein support a role for Acrp30 protein as a factor in the system of energy balance or homeostasis involving food intake, and carbohydrate and lipid catabolism and anabolism. Thus, the ability to modify or control the expression and activity of Acrp30 allows for methods of altering the energy balance (e.g., nutritional status) of a vertebrate, particularly a mammal such as a human. In particular, the present invention allows for treatment of a variety of conditions involving the energy balance (e.g., nutritional status, lipid deposition) of a host (e.g., vertebrate, particularly mammal such as a human), such as obesity, obesity related disorders and anorexia.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is the nucleotide sequence (SEQ. ID NO: 1) and amino acid sequence (SEQ ID NO:2) of murine Acrp30.

Figure 2 is an illustration of the predicted structure of the Acrp30.

Figure 3 is an alignment of the amino acid sequences of Acrp30 (SEQ ID No: 2), Hib27 (SEQ ID No: 3), Clq-C (SEQ ID No: 4) and the globular domain of the type X collagen (SEQ ID No: 5).

Figure 4 are graphs of time versus % Acrp30 or adipsin protein secreted by 3T3-L1 adipocytes in the presence (closed squares) and absence (open squares) of insulin.

Figure 5 is the nucleotide sequence (SEQ ID NO: 6) and amino acid sequence (SEQ ID NO: 7) of human Acrp30.

Figure 6 is a comparison of the amino acid sequence of the mouse Acrp30 (SEQ ID No: 2) and the amino acid sequence of the human Acrp30 (SEQ ID No: 7).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery of a novel 30 kD secretory protein, termed adipocyte complement related protein (Acrp30), which is made exclusively in adipocytes. Adipocytes also secrete tumor necrosis factor  $\alpha$ , (TNF $\alpha$ ), complement factors C3 and B (Hotamisligil, G.S., et al., *Science* 250:87-91 (1993); Flier, J.S., et al., *Science* 237:405-8 (1987), adipsin and the ob gene product.

As shown herein, Acrp30 participates in the delicately balanced system of energy homeostasis involving food intake and carbohydrate and lipid catabolism. Experiments described herein further corroborate the existence of an insulin-regulated secretory pathway for adipocytes. In particular, the data described herein demonstrates that Acrp30 and serum insulin mutually counterregulate each other.



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Acrp30 is structurally similar to complement factor C1q and to a hibernation-specific protein isolated from the plasma of Siberian chipmunks. Acrp30 is an abundant serum protein and, like adipsin, secretion of Acrp30 by adipocytes is initially enhanced as a result of exposure of adipocytes to insulin. Subsequently (after exposure of adipocytes to insulin for a longer period) adipocyte secretion of Acrp30 is inhibited. As Acrp30 activity decreases, insulin levels increase. The data provided herein show that, like the ob protein, Acrp30 is a factor that is involved in the control of the energy balance (e.g., energy metabolism, nutritional state, lipid storage) of a vertebrate (e.g., mammal).

The subject invention relates to DNA encoding vertebrate Acrp30 protein, (e.g., mammalian) particularly mammalian Acrp30 protein, such as rodent and human Acrp30. The DNA of the present invention includes DNA encoding murine Acrp30 (SEQ ID NO: ), DNA encoding human Acrp30 (SEQ ID NO:6), DNA encoding other vertebrate Acrp30 and portions thereof which either encode vertebrate Acrp30 or which are characteristic of Acrp30 encoding DNA and can be used to identify nucleotide sequences which encode Acrp30 (e.g., a nucleic acid probe), as well as to complements of the foregoing sequences.

Identification of Acrp30 makes it possible to isolate DNA encoding Acrp30 from other vertebrate organisms (e.g., monkey, pig) using nucleic acid probes which hybridize to all or a portion of the nucleotide sequences described herein and known hybridization methods. For example, as described in Example 5, the murine Acrp30 nucleotide sequence was used to produce a probe for isolation of the human homologue of Acrp30 using a hybridization method. Such nucleic acids can be detected and isolated under high stringency conditions or moderate stringency conditions, for example. "High stringency conditions" and "moderate

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stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. et al., eds., Vol. 1, Suppl. 26, 1991), the teachings of which are hereby incorporated by reference. Factors such as probe length, base composition, percent mismatch between the hybridizing sequences, temperature and ionic strength influence the stability of nucleic acid hybrids. Thus, high or moderate stringency conditions can be determined empirically, depending in part upon the characteristics of the known DNA to which other unknown nucleic acids are being compared for sequence similarity. See Maniatis et al., *Molecular Cloning, A Laboratory Manual*, 2d, Cold Spring Harbor Press (1989) which is incorporated herein by reference.

The invention also includes products encoded by the DNA described herein. In one embodiment, the invention relates to RNA transcribed from the nucleotide sequences of Acrp30.

In another embodiment, the invention relates to Acrp30 encoded by the nucleotide sequences described herein. The present invention relates to isolated, recombinantly produced or synthetic (e.g., chemically synthesized) Acrp30 of vertebrate origin (e.g., mammalian), particularly of rodent and human origin. The Acrp30 of the present invention has the amino acid sequence of SEQ ID No: 2, the amino acid sequence of SEQ ID No: 7, amino acid sequences which encode other vertebrate Acrp30 and portions thereof which encode Acrp30.

This invention includes portions of the above mentioned DNA, RNA and proteins. As used herein, "portion" refers to portions of sequences, proteins and substances of sufficient size or sequence to have the function or activity of Acrp30 involved in the nutritional status of the organism or mammal (e.g., a protein that is expressed

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by adipocytes, exhibits altered (e.g., enhanced or inhibited) secretion by insulin, and is present in normal serum). In addition, the terms include a nucleotide sequence which, through the degeneracy of the genetic code, encodes the same peptide as a peptide whose sequence is presented herein (SEQ ID NO:2, SEQ ID NO:7). The nucleic acid or protein described herein may also contain a modification of the molecule such that the resulting gene produced is sufficiently similar to that encoded by the unmodified sequence that it has essentially the same activity. An example of such a modification would be a "silent" codon or amino acid substitution, for instance, from one acidic amino acid to another acidic amino acid, or from one codon encoding a hydrophobic amino acid to another codon encoding a hydrophobic amino acid. See Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Greene Publ. Assoc. and Wiley-Interscience 1989.

The claimed DNA, RNA and proteins described herein refer to substantially pure or isolated nucleic acids and proteins, which can be isolated or purified from vertebrate sources, particularly mammalian (e.g., human, murine) sources in which they occur in nature, using the sequences described herein and known methods. In addition, the claimed DNA, RNA and proteins of the present invention can be obtained by genetic engineering (i.e., are recombinantly produced) or by chemical synthesis using the sequences described herein and known methods.

The present invention also relates to expression vectors comprising DNA encoding Acrp30 of vertebrate origin, particularly rodent and human DNA encoding Acrp30. In particular embodiments, the expression vectors of the present invention comprise DNA having the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 6 or portions thereof. The construction of expression vectors can be accomplished using known genetic engineering techniques or by using

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commercially available kits. (See, e.g., Sambrook, J., et al., Molecular Cloning 2nd Ed. Cold Spring Harbor Press, 1989; Ausubel, F.M., et al., Current Protocols In Molecular Biology, Green-Publishing Assoc. and Wiley-Interscience, 5 1988).

Also encompassed by the present invention is an agent which interacts with Acrp30, directly or indirectly, and alters its activity. In one embodiment, the agent is an inhibitor of Acrp30. Inhibitors of Acrp30 include 10 substances which inhibit expression, function or activity of Acrp30 directly or indirectly (e.g., expression by adipocytes, altered secretion in response to insulin and presence in serum). The embodiment which encompasses inhibitors of Acrp30 includes antibodies directed against 15 or which bind to Acrp30, including portions of antibodies, which can specifically recognize and bind to Acrp30. The term "antibody" includes polyclonal and monoclonal antibodies, as well as single chain antibodies, chimeric or humanized antibodies. The antibody preparations include 20 antibodies which are monospecific for mammalian, particularly human and murine, Acrp30. Preparation of antibody can be performed using the encoded protein of this invention and any suitable procedure. A variety of methods is described in the following publications, the teachings 25 of which are incorporated by reference: (Harlow, E., et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988; Huse, W.D., et al., Journal of Science 246:1275-1281 (1989); Moore, J.P., Journal of Clinical Chemistry 35:1849-1853 (1989) Kohler et al., 30 Nature, 256:495-497 (1975) and Eur. J. Immunol. 6:511-519 (1976); Milstein et al., Nature 266:550-552 (1977); Koprowski et al., U.S. Patent No. 4,172,124; Harlow, E. and D. Lane, 1988, Antibodies: A Laboratory manual, (Cold Spring Harbor Laboratory: Cold Spring Harbor, NY); Current 35 Protocols In Molecular Biology, Vol. 2 (Supplement 27,

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Summer '94), Ausubel, F.M. et al., Eds., (John Wiley & Sons: New York, NY), Chapter 11; (1991)).

Alternatively, an inhibitor can be an agent other than an antibody (e.g., small organic molecule, protein, peptide) which binds Acrp30 and directly blocks its activity. The inhibitor can be an agent which mimics Acrp30 structurally but lacks its function or can be an agent which binds or interacts with a molecule which Acrp30 normally binds or interacts with, thus blocking Acrp30 from doing so and preventing it from exerting the effects it would normally exert. An inhibitor of Acrp30 can be a substance which inhibits the expression of Acrp30 by adipocytes or the ability of insulin to alter the secretion of Acrp30 from adipocytes. An inhibitor can be DNA or RNA which binds DNA encoding Acrp30 or Acrp30 RNA and prevents its translation or transcription, thus reducing Acrp30 expression.

In another embodiment, the agent is an enhancer of Acrp30. An enhancer of Acrp30 is an agent which increases the activity of Acrp30 (increases the effect of a given amount or level of Acrp30), increases the length of time it is effective (by preventing its degradation or otherwise prolonging the time during which it is active) or both.

Enhancers of Acrp30 also include substances which enhance the expression, function or activity of Acrp30. For example, expression vectors comprising a nucleotide sequence encoding Acrp30 can be administered to a host to enhance expression of Acrp30 in the host. In addition, insulin can be administered to a host to alter the secretion of Acrp30 in the host.

The present invention also relates to a method of identifying a substance or agent which is an inhibitor or an enhancer of Acrp30. The agent to be assessed is combined with Acrp30 and a molecule (i.e., the molecule) which Acrp30 normally interacts with or binds. If Acrp30

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is unable to interact with or bind the molecule in the presence of the agent when compared to a control test sample which does not contain the agent (i.e., a test sample containing Acrp30 and the molecule) then the agent is an inhibitor. Alternatively, if interaction with or binding of Acrp30 with the molecule is increased or enhanced in the presence of the agent to be assessed when compared to a control test sample, then the agent is an enhancer of Acrp30.

10 Several expression vectors for use in making the constructs described herein and administering Acrp30 to a host are available commercially or can be produced using known recombinant DNA and cell culture techniques. For example, vector systems such as retroviral, yeast or  
15 vaccinia virus expression systems, or virus vectors can be used in the methods and compositions of the present invention (Kaufman, R.J., *J. of Method. in Cell. and Molec. Biol.*, 2:221-236 (1990)). Other techniques using naked plasmids of DNA, and cloned genes encapsidated in liposomes  
20 or in erythrocyte ghosts, can be used to introduce the constructs of the present invention into a host (Freidman, T., *Science*, 244:1275-1281 (1990); Rabinovich, N.R. et al., *Science*, 265:1401-1404 (1994)).

The Acrp30 nucleic acids (DNA, RNA) and protein  
25 products of the present invention can be used in a variety of ways. In one embodiment, the sequences described herein can be used to detect Acrp30 in a sample. For example, a labeled nucleic acid probe having all or a functional portion of the nucleotide sequence of mammalian Acrp30 can  
30 be used in a method to detect mammalian Acrp30 in a sample. In one embodiment, the sample is treated to render nucleic acids in the sample available for hybridization to a nucleic acid probe. The resulting treated sample is combined with a labeled nucleic acid probe having all or a  
35 portion of the nucleotide sequence of mammalian Acrp30,

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under conditions appropriate for hybridization of complementary sequences to occur. Detection of hybridization of the sample with the labeled nucleic acid probe indicates the presence of mammalian Acrp30 in a sample. In addition, this embodiment provides a means of identifying adipocytes in a sample. As described herein, Acrp30 is produced exclusively in adipocytes. Thus, detecting the presence of Acrp30 in a sample using this embodiment is also an indication that the sample contains adipocytes.

Alternatively, a method of detecting mammalian Acrp30 in a sample can be accomplished using an antibody directed against Acrp30 or a portion of mammalian Acrp30. Detection of specific binding to the antibody indicates the presence of mammalian Acrp30 in the sample (e.g., ELISA). This could reflect a clinically relevant condition associated with Acrp30.

In addition, an antibody directed against Acrp30 can be used to determine the presence of adipocytes in cells, such as in cultured cells and in samples obtained from individuals. For example, primary cells derived from a tissue sample are cultured in appropriate cell culture medium. A sample of conditioned culture medium (i.e., medium which has been exposed to the cells of the primary culture for a period of time) can be removed and tested for the presence of Acrp30 using an antibody directed against Acrp30. Detection of specific binding of the antibody indicates the presence of Acrp30 in the conditioned culture medium, which indicates that adipocytes are present in the cultured cells.

The sample for use in the methods of the present invention includes a suitable sample from a vertebrate (e.g., mammal, particularly human). For example, the sample can be cells, blood, urine, lymph or tissue from a mammal.

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The present invention also relates to a method of regulating or altering the energy balance (e.g., nutritional status, lipid deposition) of a host (e.g., mammal) by administering to the host an agent which interacts with Acrp30 directly or indirectly. For example, in the instance in which weight loss is desired (e.g., obesity), an inhibitor or an enhancer of Acrp30 (e.g., an antibody which binds to Acrp30) can be administered to a mammal to control weight gain in the mammal. In the instance in which weight gain is desired (e.g., anorexia), an inhibitor or enhancer of Acrp30 (e.g., insulin, expression vectors comprising nucleotide sequences encoding Acrp30) can be administered to a mammal to enhance weight gain in the mammal.

The following is a description of the isolation and characterization of Acrp30. As described in Example 1, in order to identify novel adipocyte-specific proteins, portions of 1000 clones from a subtractive cDNA library enriched in mRNAs induced during adipocyte differentiation of 3T3-L1 fibroblasts were randomly sequenced. Northern blot analysis using one ~250 bp clone showed a marked induction during adipocyte differentiation and thus a full-length cDNA was isolated and sequenced. The encoded protein, Acrp30, is novel; it contains 247 amino acids with a predicted molecular weight of 28 kD. Acrp30 consists of a predicted amino-terminal signal sequence, followed by a stretch of 27 amino acids that does not show any significant homology and then by 22 perfect GlyXPro or GlyXX repeats (Figures 1 and 2). As shown in Figure 3, the carboxy-terminal globular domain exhibits striking homology to a number of proteins, such as the globular domains of type VIII and type X collagens (i.e., coll type x) (Reichenberger, E., et al., *Febs. Lett.*, 311:305-10 (1992)), the subunits of complement factor C1q (i.e., C1q.c) (Reid, K.B., et al., *Biochem. J.*, 203:559-69 (1982))



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and a protein found in the serum of hibernating animals during the summer months (i.e., Hib27) (Kondo, N. & Kondo, J., *J. Biol. Chem.*, 267:473-8 (1992)). Structurally, albeit not at the primary sequence level, the protein

5 resembles the lung surfactant protein (Floros, J., et al., *J. Biol. Chem.*, 261:9029-33 (1986)) and the hepatocyte mannan-binding protein (Drickamer, K., et al., *J. Biol. Chem.*, 261:6878-87 (1986)), both of which have collagen-like domains and globular domains of similar size.

10 Northern blot analysis shows that Acrp30 is expressed exclusively in adipocytes (see Example 1). It is not expressed in 3T3-L1 fibroblasts, and is induced over 100-fold during adipocyte differentiation. Induction occurs between days 2 and 4, at the same time as other adipocyte-specific proteins such as GLUT4 (Charron, M.J., et al., *Proc. Natl. Acad. Sci. USA*, 86:2535-9 (1989)) and Rab3D (Baldini, G., et al., *Proc. Natl. Acad. Sci. USA*, 89:5049-52 (1992)).

As described in Example 2, an antibody raised against

20 a peptide corresponding to the unique amino-terminal domain of Acrp30 recognized a 3T3-L1 adipocyte protein of approximately 28 kD. Acrp30 contains one potential N-glycosylation site, within the collagen domain, but apparently is not glycosylated; Endo H treatment did not

25 cause a shift in molecular weight of Acrp30 at any time during a metabolic pulse-chase experiment. Acrp30 does become modified posttranslationally, since after 20 min. of chase there was a small but reproducible reduction in gel mobility. This most likely represents hydroxylation of

30 collagen-domain proline residues in the endoplasmic reticulum or Golgi compartments, by analogy to a similar modification in the structurally related mannan-binding protein (MBP) (Colley, K.J. and Baenziger, J.U., *J. Biol. Chem.*, 262:10290-5 (1987)). In 3T3-L1 adipocytes

35 unstimulated by insulin, 50% of newly-made Acrp30 is

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secreted into the medium at 2.5 to 3 hours of chase. Indeed, Acrp30 can be detected by Western blotting in normal mouse serum. The antipeptide antibody is specific for the mouse homologue, as it does not cross-react with ~~5~~ bovine, human or rabbit serum. As further indicated in Example 6, muscle tissue is a target organ for Acrp30 action.

Insulin causes translocation of several receptor proteins from intracellular membranes to the plasma ~~10~~ membrane (Corvera, S., et al., *J. Biol. Chem.*, 264:10133-8 (1989); Davis, R.J., et al., *J. Biol. Chem.*, 261:8708-11 (1986)). ~~Adipocytes are highly responsive to insulin and~~ translocate intracellular glucose transporters to the cell surface upon stimulation with insulin (Simpson, I.A. & ~~15~~ Cushman, S.W., *Ann. Rev. Biochem.*, 55:1059-89 (1986); Wardzala, L.J., et al., *J. Biol. Chem.*, 259:8378-83 (1984)). Insulin also causes a two-fold stimulation of adipsin secretion (Kitagawa, K., et al., *Biochim. Biophys. Acta.*, 1014:83-9 (1989)). For example, insulin stimulation ~~20~~ of adipocytes causes exocytosis of intracellular vesicles containing the GLUT4 glucose transporter and a concomitant increase in glucose uptake. Adipocytes stimulated by insulin respond initially by increased secretion of Acrp30. After an initial period of enhanced Acrp30 secretion, ~~25~~ Acrp30 secretion decreases and returns to levels secreted by adipocytes not stimulated by insulin. As described in the pulse chase experiment of Example 3, during the first 60 minutes of chase, insulin causes a four-fold increase in secretion of newly-made Acrp30. After 60 minutes the rates ~~30~~ of Acrp30 secretion are the same in unstimulated and insulin-stimulated cells. Similarly, insulin causes a four-fold increase in adipsin secretion during the first 30 minutes of chase, but afterwards the rate of adipsin secretion is the same in control and insulin-treated cells. ~~35~~ See Figure 4. (Kitagawa, K., et al., *Biochim. Biophys.*

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Acta., 1014:83-9 (1989)). It is reasonable to expect that a fraction of newly-made adipsin and Acrp30 are sorted, probably in the trans-Golgi reticulum, into regulated secretory vesicles whose exocytosis is induced by insulin whereas the balance is sorted into vesicles that are constitutively exocytosed. Partial sorting of protein hormones into regulated secretory vesicles has been seen in other types of cultured cells (Moore, H.-P.H., et al., *Nature*, 302:434-436 (1983); Sambanis, A., Stephanopoulos, G., et al., *Biotech. Bioeng.*, 35:771-780 (1990)).

Chronic or longer term exposure of adipocytes to insulin inhibits expression of Acrp30, both at the level of mRNA and protein. As described in Example 7, Acrp30 represses (inhibits) insulin levels and insulin represses Acrp30 levels. Thus, insulin and Acrp30 are part of a feedback loop that maintains constant levels of both of these agonists.

Complement factor C1q consists of three related polypeptides that form heterotrimeric subunits containing a three-stranded collagen "tail" and three globular "heads"; six of these subunits generate an eighteen-mer complex often referred to as a "bouquet of flowers." The experiments described in Example 4 show that Acrp30 has a similar oligomeric structure, but is composed of a single type of polypeptide chain. When analyzed by velocity gradient sedimentation analysis, Acrp30 in blood serum migrates as two species of apparent molecular weights 90 kDa and 300 kDa. Disregarding the presumably non-globular shape of the complex that could lead to a slight distortion of the molecular weight determination, the former is probably a trimer and the latter could be a nonamer or dodecamer.

Isoelectric focusing followed by SDS-PAGE of [<sup>35</sup>S] Acrp30 secreted by 3T3-L1 adipocytes reveals only a single

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polypeptide, suggesting that Acrp30 forms homo-oligomeric structures. Chemical crosslinking using low concentrations of BS<sup>3</sup> of [<sup>35</sup>S] medium from 3T3-L1 adipocytes, followed by specific immunoprecipitation and SDS-PAGE under reducing conditions, shows mainly dimers and trimers. Larger concentrations of the BS<sup>3</sup> cross-linking agent generated Acrp30 proteins that migrated as hexamers as well as yet larger species. As extensively cross-linked proteins migrate aberrantly upon SDS-PAGE, it is difficult to determine the exact size of the high molecular weight form. It could represent either a nonamer or a dodecameric structure. Results show that Acrp30 forms homotrimers that interact to generate nonamers or dodecamers. Non-reducing SDS-PAGE reveals that two of the subunits in a trimer are disulfide-bonded together, similar to other proteins containing a collagen domain, including the macrophage scavenger receptor (Resnick, D., et al., *J. Biol. Chem.*, 268:3538-3545 (1993)). Besides being a homo-oligomer, Acrp30 differs from Clq in containing an uninterrupted stretch of 22 perfect GlyXX repeats; this suggests that Acrp30 has a straight collagen stalk as opposed to the characteristic kinked collagen domain in Clq caused by imperfect GlyXX repeats in two of the three subunits (reviewed in (Thiel, S. and Reid, K.B., *Febs. Lett.*, 250:78-84 (1989))).

The human Acrp30 protein was isolated through the use of a probe derived from the mouse Acrp30 nucleotide sequence, and sequenced, as described in Example 5. Comparison of the mouse Acrp30 amino acid sequence with the human Acrp30 amino acid sequence showed that 82% homology exists between the two sequences and that the highest degree of sequence divergence occurs near the N-terminus of the mouse and the human Acrp30 sequence.

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Acrp30 is a relatively abundant serum protein, accounting for up to 0.05% of total serum protein as judged by quantitative Western blotting using recombinant ACRP30 as a standard. Possibly Acrp30, like C3 complement released by adipocytes, is converted proteolytically to a bioactive molecule.

The experiments described herein corroborate the existence of a regulated secretory pathway in adipocytes. Whether adipsin and/or Acrp30 are in the same intracellular vesicles that contain GLUT4 and that fuse with the plasma membrane in response to insulin or are in different types of vesicles is not yet known. Adipocytes express two members of the Rab3 family, Rab3A and Rab3D (Baldini, G., et al., *Proc. Natl. Acad. Sci. USA* (1995)). These are found in vesicles of different density. Rab3s are small GTP-binding proteins involved in regulated exocytic events. Rab3A is found only in adipocytes and neuronal and neuroendocrine cells; in neurons Rab3A is localized to synaptic vesicles and is important for their targeting to the plasma membrane. It is possible that in adipocytes, Rab3A is localized to vesicles containing Acrp30 and/or adipsin and that Rab3D mediates insulin-triggered exocytosis of vesicles containing GLUT4.

The coding sequence of Acrp30, a novel serum protein which is involved in the regulatory pathway of adipocytes is now available and, as a result, compositions (e.g., nucleotide sequences, protein, expression vectors and inhibitors), methods of detecting Acrp30 and methods of inhibiting the activity of Acrp30 using all or portions of the Acrp30 DNA or encoded product (e.g., protein, RNA) are within the scope of the present invention.

The invention is further illustrated in the following examples, which are not intended to be limiting.

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Example 1 Isolation and sequencing of the murine Acrp30 protein

A full-length cDNA library templated by mRNA from 3T3-L1 adipocytes at day 8 of differentiation (Baldini, G., et al., *Proc. Natl. Acad. Sci. USA*, 89:5049-52 (1992)) was screened with a digoxigenin-labeled cDNA fragment obtained from the random sequencing screen. Labeling, hybridization, and detection were performed according to the manufacturer's instructions (Boehringer Inc.). One of the positive clones obtained was subjected to automated sequencing on an Applied Biosystems 373-A sequencer. The entire 1.3 kb insert was sequenced at least 2 independent times on one stand and once on the complementary strand. Sequence analysis was performed with the DNASTar package and showed an open reading frame of 741 bp encoding a protein of 28 kD. Homology searches were performed at NCBI using the BLAST network service, and alignments were performed with the Megalign program from DNASTar using the Clustal algorithm. Only the globular domain for the type X collagen was used for the alignment (residues 562-680).

Figure 2 is the predicted structure of murine Acrp30. The protein consists of an amino-terminal signal sequence (SS) followed by a sequence of 27 amino acids lacking significant homologies to any entries in the Genbank database. A peptide corresponding to part of this sequence, was used to generate specific anti-Acrp30 antibodies (MAP technology, Research Genetics). This region is followed by a stretch of 22 collagen repeats with 7 "perfect" Gly-X-Pro repeats (dark hatched boxes) clustered at the beginning and end of the domain interspersed with 15 "imperfect" Gly-X-Y repeats (light hatched boxes). The C-terminal 138 amino acids probably form a globular domain.

Figure 3 shows the alignment of the amino acid sequences of Acrp30 (SEQ ID NO: 2); Hib27 (SEQ ID NO: 3), a

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member of the hibernation-specific protein family; Clq-C (SEQ ID NO: 4), one of the subunits of complement Clq; and the globular domain of the type X collagen (SEQ ID NO: 5). Conserved residues are shaded. For simplicity, the other members of each family are not shown, but shaded conserved residues are in most instances conserved within each protein family.

#### Northern blot analysis of Acrp30 expression.

Isolation of mRNA from tissues and from 3T3-L1 cells at various stages of differentiation was as described in (Baldini, G., et al., *Proc. Natl. Acad. Sci. USA*, 89:5049-52 (1992)), as was [<sup>35</sup>P] labeling of DNA, agarose gel electrophoresis of mRNA, and its transfer to nylon membranes. Hybridizations were performed overnight at 42°C in 50% formamide, 5x SSC, 25 mM Na-phosphate pH 7.0, 10x Denhardt's solution, 5 mM EDTA, 1% SDS, and 0.1 mg/ml PolyA; the [<sup>35</sup>P] DNA probes were used at concentrations of 2x10<sup>6</sup> cpm/ml. The filters were subsequently washed in 2x SSC/0.1% SDS and 0.1x SSC/0.1% SDS at 50°C. The same filters were thereafter stripped and reprobed with a probe encoding one of the constitutively expressed cytosolic hsp70s. Autoradiography was for 4 hours (Acrp30) and 24 hours (hsp70).

Northern blot analysis of Acrp30 expression in murine cells from kidney, liver, brain, testis, fat, (adipocytes) diaphragm, heart, lung, spleen and cultured 3T3-L1 adipocytes was carried out. PolyA-RNA isolated from various tissues was probed with the full-length Acrp30 cDNA. The predominant Acrp30 mRNA is 1.4kb and was shown to be expressed only in adipose tissue and cultured 3T3-L1 adipocytes. Overexposure of the autoradiogram did not reveal expression in any other tissue.

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Induction of the Acrp30 message during differentiation of 3T3-L1 fibroblasts to adipocytes was assessed.

Induction of Acrp30 occurs primarily between days 2 and 4 of differentiation, the same time at which induction of the insulin receptor and the insulin-responsive glucose transporter GLUT4 occurs.

Example 2 Acrp30 is a secretory protein found in blood

Ten 6 cm diameter dishes of 3T3-L1 adipocytes were starved for 30 min. in Dulbecco's modified Eagle medium (DME, ICN, Costa Mesa), lacking cysteine and methionine and then labeled for 10 min. in the same medium containing 0.5 mCi/ml of Express Protein Labeling Reagent (1000 Ci/mmol) [NEN (Boston, MA)]. The cells were then washed twice with DME supplemented with unlabeled cysteine and methionine and then fresh growth medium containing 300  $\mu$ M cycloheximide was added. At each of the indicated time points the medium from one plate was collected and the cells washed with ice-cold PBS and then lysed in lysis buffer (1% Triton X-100, 60 mM octyl-glucoside, 150 mM NaCl, 20 mM Tris pH 8.0, 2 mM EDTA, 1 mM PMSF, and 2  $\mu$ g/ml leupeptin). Insoluble material from both the medium and cell lysate was removed by centrifugation (15,000g for 10 min.); the supernatants were precleared with 50  $\mu$ l Protein A-Sepharose for 30 min. at 4°C and then immunoprecipitated with 50  $\mu$ l of affinity-purified anti-Acrp30 antibody for 2 hrs. at 4°C. Immunoprecipitates were washed 4 times in lysis buffer lacking octylglucoside and once in PBS, then resuspended in Endo H buffer (0.1 M Na-citrate pH 6.0, 1% SDS), boiled for 5 min., and intracellular samples were incubated for 2 hrs. either in absence (-) or presence (+) of 1000 U Endo H (New England Biolabs) at 37°C. Reactions were stopped by boiling in 2X sample buffer (250 mM Tris pH 6.8, 4mM EDTA, 4% SDS, 20% sucrose) and analyzed by electrophoresis through a 12% polyacrylamide gel containing SDS. Mr:



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Molecular weight marker. Labeled proteins were visualized by fluorography.

Specific anti-Acrp30 antibodies raised against a peptide corresponding to the unique amino-terminal sequence domain of Acrp30 (EDDVTTTEELAPALV, residues (18-32) SEQ ID NO: 8) which was generated in rabbits, recognized a 3T3-L1 adipocyte protein of approximately 28 kD. Acrp30 contains one potential N-glycosylation site, within the collagen domain, but apparently is not glycosylated; Endo H treatment did not cause a shift in molecular weight of Acrp30 at any time during a metabolic pulse-chase experiment. Acrp30 does become modified posttranslationally, since after 20 min. of chase there was a small but reproducible reduction in gel mobility. This most likely represents hydroxylation of collagen-domain proline residues in the endoplasmic reticulum or Golgi compartments, by analogy to a similar modification in the structurally related mammalian-binding protein (MBP) (Colley, K.J. and Baenziger, J.U., *J. Biol. Chem.*, 262:10290-5 (1987)). In 3T3-L1 adipocytes unstimulated by insulin, 50% of newly-made Acrp30 is secreted into the medium at 2.5 to 3 hours of chase.

#### Western blot analysis.

One microliter of fetal calf, rabbit, mouse and human serum was boiled for 5 min. in 2X sample buffer and analyzed by SDS-PAGE and Western blotting with the anti-Acrp30 antibody according to standard protocols. Antibody was visualized with an anti-rabbit IgG antibody coupled to horseradish peroxidase using a chemiluminescence kit from New England Nuclear Corporation, Boston.

Results showed that Acrp30 was detected by Western blotting in serum from mice; the antibody does not crossreact with calf, human or rabbit serum.

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Example 3 Insulin stimulation of Acrp30 and Adipsin  
secretion by 3T3-L1 adipocytes

Two 10 cm dishes of 3T3-L1 adipocytes on the 8th day after differentiation were labeled for 10 min. in medium containing [<sup>35</sup>S] methionine and cysteine as described in Example 2. The cells were then incubated in growth medium containing cycloheximide and containing or lacking 100 nM insulin. Every 30 min. the culture medium was removed and replaced with fresh, prewarmed medium containing or lacking 100 nM insulin. The media were subjected to sequential immunoprecipitations with anti-Acrp30 and anti-adipsin antibodies as described in Example 2 and analyzed by electrophoresis through a 12% polyacrylamide gel containing SDS. Acrp30 and adipsin contain a comparable number of cysteine and methionine residues (7 and 9, respectively) and equal exposures of the autoradiograms were used. Therefore, one can determine from the intensities of the bands resulting from the 12% polyacrylamide gel containing SDS that approximately equal amounts of the two proteins are secreted. As judged by the amount of [<sup>35</sup>S] proteins remaining in the cells after the 2 hr. chase, all of the [<sup>35</sup>S] adipsin and about 40% of the [<sup>35</sup>S] Acrp30 has been secreted at this time.

The autoradiograms were scanned in a Molecular Dynamics densitometer, and the cumulative amount secreted at each time point was plotted. The amount of each protein secreted after 120 min. in the presence of insulin was taken as 100%. Figure 4 shows quantitation of Acrp30 and Adipsin secretion by 3T3-L1 adipocytes in the presence (closed squares) and absence (open circles) of insulin.

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Example 4 Oligomeric structure of Acrp30

One 10 cm plate of 3T3-L1 adipocytes on the 8th day after differentiation was labeled overnight with [<sup>35</sup>S] methionine and cysteine as described in Example 2. The medium was collected and, by means of several spins in a Centricon 10 microconcentrator, the buffer was replaced with 150 mM NaCl, 50 mM KPi, pH 8.5. A stock solution of 200 mg/ml Bis (sulfosuccinimidyl) suberate (BS<sup>3</sup>; Pierce Inc.) in dimethylsulfoxide was prepared and added to the indicated final concentrations. Reactions were allowed to proceed for 30 min. on ice and excess crosslinker was quenched by addition of 500 mM Tris buffer, pH 8.0.

Samples were diluted 1:1 with lysis buffer and subjected to immunoprecipitation with anti-Acrp30 antibodies.

Immunoprecipitates were analyzed by gradient SDS-PAGE (7-12.5% acrylamide) followed by fluorography. In the lane "Total" 1% of the amount of cell medium used for the cross-linking reactions was analyzed on the same gel; a comparison of the "Total" lane and lane 1 demonstrates the specificity of the antibody used for immunoprecipitation. Rainbow markers (Amersham) together with a Phosphorylase b ladder (Sigma) were used as molecular weight markers.

[<sup>35</sup>S] labeled 3T3-L1 culture supernatant was incubated with increasing amounts of the BS<sup>3</sup> crosslinking reagent and immunoprecipitated with Acrp30-specific antibodies. The results revealed a set of crosslinked products whose molecular sizes are multiples of 30 kDa. Predominant species are trimers, hexamers and a high molecular weight species (asterisk) that could correspond to a nonamer or a dodecamer.

Medium from 3T3-L1 adipocytes on the 8th day after differentiation labeled overnight with [<sup>35</sup>S] methionine and cysteine was immunoprecipitated with anti-Acrp30 antibodies as described in Example 2. ~Half of the sample was

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subjected to SDS-PAGE (7-12.5% acrylamide gradient) in the presence (reducing) or absence (non-reducing) of 50 mM DTT. Labeled proteins were detected by fluorography.

- One microliter of mouse serum was diluted with 50  $\mu$ l PBS and layered on top of a 4.5 ml. linear 5-20% sucrose gradient in PBS and centrifuged for 10 hrs. at 60,000 rpm in a SW60 rotor of a Beckman ultracentrifuge. Thirteen 340  $\mu$ l fractions were collected from the top and analyzed by SDS-PAGE and Western blotting using anti-Acrp30 antibodies.
- 10 An identical gradient was run in parallel with a set of molecular weight standards: cytochrome c (14kD), carbonic anhydrase (29 kD), bovine serum albumin (68 kD), alcohol dehydrogenase (150 kD),  $\beta$ -amylase (200 kD), and apoferritin (443 kD). Results show that Acrp30 forms homotrimers that
- 15 interact together to generate nonamers or dodecamers. Non-reducing SDS-PAGE reveals that two of the subunits in a trimer are disulfide-bonded together, similar to other proteins containing a collagen domain, including the macrophage scavenger receptor (Resnick, D., et al., *J. Biol. Chem.*, 268:3538-3545 (1993)).
- 20

Velocity gradient centrifugation of mouse serum displays two discrete Acrp30-immunoreactive species. The smallest corresponds to a trimer of Acrp30 polypeptides and the larger a nonamer or dodecamer.

25 Example 5 Isolation and sequencing of the human Acrp30 protein

- The sequencing and isolation of the human Acrp30 protein was performed using methods similar to those described in Example 1. The nucleotide sequence of human
- 30 Acrp30 is shown in Figure 5. Figure 6 illustrates a comparison of the mouse and human Acrp30 sequences.

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Southern Blot Analysis:

The complete mouse cDNA was used as a probe for a low stringency hybridization on genomic DNA from a number of different species: mouse, human, Drosophila and Xenopus samples were tested. Crosshybridizing bands were detected in the human sample; no signal was seen in the Drosophila and Xenopus samples. The mouse cDNA probe was labeled according to standard methods. The probe was used at  $2 \times 10^6$  cpm/ml. Hybridizations were performed overnight at  $42^\circ$  in 30% formamide, 5xSSC, 25 mM Na-phosphate pH 7.0, 10x Denhardt's solution, 5 mM EDTA, 1% SDS, and 0.1 mg/ml PolyA. The filters were subsequently washed in 2x SSC/0.1% SDS at  $50^\circ\text{C}$ .

Isolation of clone:

The conditions established for Southern blot analysis were used to screen for the human homolog. [A reduction of 20% formamide during the hybridization (30% instead of the standard 50% in high stringency hybridizations) translates into a reduction of  $14^\circ\text{C}$  in the hybridization temperature in aqueous buffers]. Therefore, colony hybridization was performed at  $50^\circ\text{C}$  using the digoxigenin-labeled mouse cDNA fragment. Washes were done with 2x SSC/0.1% SDS at  $50^\circ\text{C}$ . All other buffers and incubations, including labeling of the mouse probe with digoxigenin and detection of positive plaques were performed as described for the isolation of the mouse clone according to the manufacturer's instructions (Boehringer Inc.). A commercially available library was used for the isolation of the human clone; a human fat cell 5'-Stretch Plus cDNA library (sold by Clontech Inc., Article #HL3016b) was used. The mRNA source for this library was abdominal fat from a Caucasian female. A total of  $5 \times 10^4$  plaques were screened and several positive clones were isolated. For one of the positive clones obtained, a series of Exonuclease III deletions was generated. These deletions were subjected to automated

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sequencing on an Applied Biosystems 373-A sequencer. Human Acrp30 is 82% similar to its mouse counterpart with the highest degree of sequence divergence located near the N-terminus.

5 Example 6 Muscle Tissue Is One Of The Target Organs For  
Acrp30 Action

As indicated in Example 2, Acrp30 is released from its unique site of synthesis in adipose tissue into the bloodstream. This raised the question of the potential  
10 target organ(s) for Acrp30 action. The data described below indicates that muscle tissue is one of the target sites for Acrp30.

Purified, radiolabeled Acrp30 injected into mice accumulated in skeletal and heart muscle. Significant  
15 levels were also found in liver, presumably due to the presence of partially denatured Acrp30 protein in the preparation. Other highly vascularized tissues, such as kidney and lung, did not accumulate notable levels. Control injections with radiolabeled transferrin gave rise  
20 to a distinct distribution of counts, underscoring the specificity of the Acrp30 accumulation in muscle tissue.

Steady state distribution of Acrp30 within the body was assessed by Western blot analysis of various tissues and indicated high levels in adipose tissue. Tissue  
25 isolation and Western Blot analysis was performed as described in Scherer, P.E., et al., *J. Cell Biol.*, 127:1233-1243 (1994). This is in agreement with previous Northern blot analysis that adipose tissue is the sole source of Acrp30 production within the body. However,  
30 significant levels of Acrp30 were also found in heart and skeletal muscle. Similarly to the injection studies described above, this did not reflect serum-borne Acrp30, since highly vascularized tissues such as liver and kidney do not display significant Acrp30 levels under these  
35 conditions.

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C2C12 cells are a tissue culture cell line that can be differentiated into skeletal myoblasts. Binding of labeled Acrp30 to this cell line increased significantly in the course of the differentiation process.

5 Example 7 Acrp30 And Serum Insulin Mutually Counterregulate  
Each Other

Injection of affinity-purified anti-Acrp30 antibodies in mice (test mice) resulted in a two-fold increase of plasma insulin levels over a period of 8 days compared to  
10 the effects of injection of an identical amount of pre-immune antibodies into control mice. Concomitantly, plasma levels of free fatty acids dropped by about 30% in test mice, compared to control mice. All other serum parameters measured, including glucose clearance, remained the same.

15 Day 8 dipocytes were washed three times in DME (Dulbecco's Modified Eagle's Medium) lacking Fetal Calf Serum. Subsequently, the cells were incubated overnight (12-15 hrs) in DME containing 1  $\mu$ M insulin or in DME lacking insulin as a control. The next day, cells were  
20 either subjected to mRNA isolation (according to standard protocols) or a pulse-chase experiment was performed as described in Scherer, P.E., et al., *J. Biol. Chem.*, 270:26746-26749 (1995).

Under the conditions used, after approximately  
25 12 hours of exposure of 3T3-L1 adipocytes to elevated levels of insulin in tissue culture, expression of Acrp30 both at the level of mRNA and protein was abolished.

Taken together, these experiments suggest that Acrp30, directly or indirectly, represses insulin levels, while  
30 insulin, directly or indirectly, represses Acrp30 levels. The data suggests that insulin and Acrp30 are part of a feedback loop that maintains constant levels of these agonists. Consequently, Acrp30 is a pharmacological target that allows modulation of insulin levels by inhibiting the

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function of Acrp30 or by regulating its expression and/or secretion from adipocytes.

#### EQUIVALENTS

Those skilled in the art will recognize, or be able to  
5 ascertain using no more than routine experimentation, many  
equivalents to the specific embodiments of the invention  
described specifically herein. Such equivalents are  
intended to be encompassed in the scope of the following  
claims.



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(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: WHI95-05A PCT  
(B) FILING DATE:  
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-32-

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1276 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 46..786

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Leu Leu	
1	
TTG CAA GCT CTC CTG TTC CTC TTA ATC CTG CCC AGT CAT GCC GAA GAT	102
Leu Gln Ala Leu Leu Phe Leu Leu Ile Leu Pro Ser His Ala Glu Asp	
5 10 15	
GAC GTT ACT ACA ACT GAA GAG CTA GCT CCT GCT TTG GTC CCT CCA CCC	150
Asp Val Thr Thr Thr Glu Glu Leu Ala Pro Ala Leu Val Pro Pro Pro	
20 25 30 35	
AAG GGA ACT TGT GCA GGT TGG ATG GCA GGC ATC CCA GGA CAT CCT GGC	198
Lys Gly Thr Cys Ala Gly Trp Met Ala Gly Ile Pro Gly His Pro Gly	
40 45 50	
CAC AAT GGC ACA CCA GGC CGT GAT GGC AGA GAT GGC ACT CCT GGA GAG	246
His Asn Gly Thr Pro Gly Arg Asp Gly Arg Asp Gly Thr Pro Gly Glu	
55 60 65	
AAG GGA GAG AAA GGA GAT GCA GGT CTT CTT GGT CCT AAG GGT GAG ACA	294
Lys Gly Glu Lys Gly Asp Ala Gly Leu Leu Gly Pro Lys Gly Glu Thr	
70 75 80	
GGA GAT GTT GGA ATG ACA GGA GCT GAA GGG CCA CGG GGC TTC CCC GGA	342
Gly Asp Val Gly Met Thr Gly Ala Glu Gly Pro Arg Gly Phe Pro Gly	
85 90 95	
ACC CCT GGC AGG AAA GGA GAG CCT GGA GAA GCC GCT TAT ATG TAT CGC	390
Thr Pro Gly Arg Lys Gly Glu Pro Gly Glu Ala Ala Tyr Met Tyr Arg	
100 105 110 115	
TCA GCG TTC AGT GTG GGG CTG GAG ACC CGC GTC ACT GTT CCC AAT GTA	438
Ser Ala Phe Ser Val Gly Leu Glu Thr Arg Val Thr Val Pro Asn Val	
120 125 130	

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CCC ATT CGC TTT ACT AAG ATC TTC TAC AAC CAA CAG AAT CAT TAT GAC Pro Ile Arg Phe Thr Lys Ile Phe Tyr Asn Gln Gln Asn His Tyr Asp 135 140 145	486
GGC AGC ACT GGC AAG TTC TAC TGC AAC ATT CCG GGA CTC TAC TAC TTC Gly Ser Thr Gly Lys Phe Tyr Cys Asn Ile Pro Gly Leu Tyr Tyr Phe 150 155 160	534
TCT TAC CAC ATC ACG GTG TAC ATG AAA GAT GTG AAG GTG AGC CTC TTC Ser Tyr His Ile Thr Val Tyr Met Lys Asp Val Lys Val Ser Leu Phe 165 170 175	582
AAG AAG GAC AAG GCC GTT CTC TTC ACC TAC GAC CAG TAT CAG GAA AAG Lys Lys Asp Lys Ala Val Leu Phe Thr Tyr Asp Gln Tyr Gln Glu Lys 180 185 190 195	630
AAT GTG GAC CAG GCC TCT GGC TCT GTG CTC CTC CAT CTG GAG GTG GGA Asn Val Asp Gln Ala Ser Gly Ser Val Leu Leu His Leu Glu Val Gly 200 205 210	678
GAC CAA GTC TGG CTC CAG GTG TAT GGG GAT GGG GAC CAC AAT GGA CTC Asp Gln Val Trp Leu Gln Val Tyr Gly Asp Gly Asp His Asn Gly Leu 215 220 225	726
TAT GCA GAT AAC GTC AAC GAC TCT ACA TTT ACT GGC TTT CTT CTC TAC Tyr Ala Asp Asn Val Asn Asp Ser Thr Phe Thr Gly Phe Leu Leu Tyr 230 235 240	774
CAT GAT ACC AAC TGA CTGCAAC TACCCATAGC CCATACACCA GGAGAATCAT His Asp Thr Asn 245	826
GGAACAGTCG ACACACTTTC AGCTTAGTTT GAGAGATTGA TTTTATTGCT TAGTTTGAGA	886
GTCCTGAGTA TTATCCACAC GTGTACTCAC TTGTTTCATTA AACGACTTTA TAAAAAATAA	946
TTTGTGTTCC TAGTCCAGAA AAAAAGGCAC TCCCTGGTCT CCACGACTCT TACATGGTAG	1006
CAATAACAGA ATGAAAATCA CATTGGGTAT GGGGGCTTCA CAATATTGCG ATGACTGTCT	1066
GGAAGTAGAC CATGCTATTT TTCTGCTCAC TGTACACAAA TATTGTTTAC ATAAACCTTA	1126
TAATGTAAAT ATGAAATACA GTGATTACTC TTCTCACAGG CTGAGTGTAT GAATGTCTAA	1186
AGACCCATAA GTATTAAAGT GGTAGGGATA AATTGGAAAA AAAAAAAAAA AAAAAGAAAA	1246
ACTTTAGAGC ACACTGGCGG CCGTTACTAG	1276

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 247 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Leu Leu Gln Ala Leu Leu Phe Leu Leu Ile Leu Pro Ser His  
 1 5 10 15  
 Ala Glu Asp Asp Val Thr Thr Thr Glu Glu Leu Ala Pro Ala Leu Val  
 20 25 30  
 Pro Pro Pro Lys Gly Thr Cys Ala Gly Trp Met Ala Gly Ile Pro Gly  
 35 40 45  
 His Pro Gly His Asn Gly Thr Pro Gly Arg Asp Gly Arg Asp Gly Thr  
 50 55 60  
 Pro Gly Glu Lys Gly Glu Lys Gly Asp Ala Gly Leu Leu Gly Pro Lys  
 65 70 75 80  
 Gly Glu Thr Gly Asp Val Gly Met Thr Gly Ala Glu Gly Pro Arg Gly  
 85 90 95  
~~Phe Pro Gly Thr Pro Gly Arg Lys Gly Glu Pro Gly Glu Ala Ala Tyr~~  
~~100 105 110~~  
 Met Tyr Arg Ser Ala Phe Ser Val Gly Leu Glu Thr Arg Val Thr Val  
 115 120 125  
 Pro Asn Val Pro Ile Arg Phe Thr Lys Ile Phe Tyr Asn Gln Gln Asn  
 130 135 140  
 His Tyr Asp Gly Ser Thr Gly Lys Phe Tyr Cys Asn Ile Pro Gly Leu  
 145 150 155 160  
 Tyr Tyr Phe Ser Tyr His Ile Thr Val Tyr Met Lys Asp Val Lys Val  
 165 170 175  
 Ser Leu Phe Lys Lys Asp Lys Ala Val Leu Phe Thr Tyr Asp Gln Tyr  
 180 185 190  
 Gln Glu Lys Asn Val Asp Gln Ala Ser Gly Ser Val Leu Leu His Leu  
 195 200 205  
 Glu Val Gly Asp Gln Val Trp Leu Gln Val Tyr Gly Asp Gly Asp His  
 210 215 220  
 Asn Gly Leu Tyr Ala Asp Asn Val Asn Asp Ser Thr Phe Thr Gly Phe  
 225 230 235 240  
 Leu Leu Tyr His Asp Thr Asn  
 245

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 185 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Thr Gln Gly Asn Pro Glu Ser Cys Asn Ala Pro Gly Pro Gln Gly  
 1 5 10 15  
 Pro Pro Gly Met Gln Gly Pro Pro Gly Thr Pro Gly Lys Pro Gly Pro  
 20 25 30  
 Pro Gly Trp Asn Gly Phe Pro Gly Leu Pro Gly Pro Pro Gly Pro Pro  
 35 40 45  
 Gly Met Thr Val Asn Cys His Ser Lys Gly Thr Ser Ala Phe Ala Val  
 50 55 60  
 Lys Ala Asn Glu Leu Pro Pro Ala Pro Ser Gln Pro Val Ile Phe Lys  
 65 70 75 80  
 Glu Ala Leu His Asp Ala Gln Gly His Phe Asp Leu Ala Thr Gly Val  
 85 90 95  
 Phe Thr Cys Pro Val Pro Gly Leu Tyr Gln Phe Gly Phe His Ile Glu  
 100 105 110  
 Ala Val Gln Arg Ala Val Lys Val Ser Leu Met Arg Asn Gly Thr Gln  
 115 120 125  
 Val Met Glu Arg Glu Ala Glu Ala Gln Asp Gly Tyr Glu His Ile Ser  
 130 135 140  
 Gly Thr Ala Ile Leu Gln Leu Gly Met Glu Asp Arg Val Trp Leu Glu  
 145 150 155 160  
 Asn Lys Leu Ser Gln Thr Asp Leu Glu Arg Gly Thr Val Gln Ala Val  
 165 170 175  
 Phe Ser Gly Phe Leu Ile His Glu Asn  
 180 185

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 246 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Val Gly Pro Ser Cys Gln Pro Gln Cys Gly Leu Cys Leu Leu  
 1 5 10 15  
 Leu Leu Phe Leu Leu Ala Leu Pro Leu Arg Ser Gln Ala Ser Ala Gly  
 20 25 30  
 Cys Tyr Gly Ile Pro Gly Met Pro Gly Met Pro Gly Ala Pro Gly Lys  
 35 40 45

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Asp Gly His Asp Gly Leu Gln Gly Pro Lys Gly Glu Pro Gly Ile Pro  
 50 55 60  
 Ala Val Pro Gly Thr Gln Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly  
 65 70 75 80  
 Met Pro Gly His Arg Gly Lys Asn Gly Pro Arg Gly Thr Ser Gly Leu  
 85 90 95  
 Pro Gly Asp Pro Gly Pro Arg Gly Pro Pro Gly Glu Pro Gly Val Glu  
 100 105 110  
 Gly Arg Tyr Lys Gln Lys His Gln Ser Val Phe Thr Val Thr Arg Gln  
 115 120 125  
 Thr Thr Gln Tyr Pro Glu Ala Asn Ala Leu Val Arg Phe Asn Ser Val  
 130 135 140  
 Val Thr Asn Pro Gln Gly His Tyr Asn Pro Ser Thr Gly Lys Phe Thr  
 145 150 155 160  
 Cys Glu Val Pro Gly Leu Tyr Tyr Phe Val Tyr Tyr Thr Ser His Thr  
 165 170 175  
 Ala Asn Leu Cys Val His Leu Asn Leu Asn Leu Ala Arg Val Ala Ser  
 180 185 190  
 Phe Cys Asp His Met Phe Asn Ser Lys Gln Val Ser Ser Gly Gly Ala  
 195 200 205  
 Leu Leu Arg Leu Gln Arg Gly Asp Glu Val Trp Leu Ser Val Asn Asp  
 210 215 220  
 Tyr Asn Gly Met Val Gly Ile Glu Gly Ser Asn Ser Val Phe Ser Gly  
 225 230 235 240  
 Phe Leu Leu Phe Pro Asp  
 245

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 132 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear.

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Pro Val Ser Ala Phe Thr Val Ile Leu Ser Lys Ala Tyr Pro Ala  
 1 5 10 15  
 Val Gly Cys Pro His Pro Ile Tyr Glu Ile Leu Tyr Asn Arg Gln Gln  
 20 25 30  
 His Tyr Asp Pro Arg Ser Gly Ile Phe Thr Cys Lys Ile Pro Gly Ile  
 35 40 45  
 Tyr Tyr Phe Ser Tyr His Val His Val Lys Gly Thr His Val Trp Val  
 50 55 60

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Gly Leu Tyr Lys Asn Gly Thr Pro Thr Met Tyr Thr Tyr Asp Glu Tyr  
 65 70 75 80  
 Ser Lys Gly Tyr Leu Asp Thr Ala Ser Gly Ser Ala Thr Met Glu Leu  
 85 90 95  
 Thr Glu Asn Asp Gln Val Trp Leu Gln Leu Pro Asn Ala Glu Ser Asn  
 100 105 110  
 Gly Leu Tyr Ser Ser Glu Tyr Val His Ser Ser Phe Ser Gly Phe Leu  
 115 120 125  
 Val Ala Pro Met  
 130

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1313 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 73..804

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGGTCGACGG TATCGATAAG CTTGATATCG AATTCCGGCT GCGGTTCTGA TTCCATACCA 60  
 GAGGGGCTCA GG ATG CTG TTG CTG GGA GCT GTT CTA CTG CTA TTA GCT 108  
 Met Leu Leu Leu Gly Ala Val Leu Leu Leu Ala  
 1 5 10  
 CTG CCC GGT CAT GAC CAG GAA ACC ACG ACT CAA GGG CCC GGA GTC CTG 156  
 Leu Pro Gly His Asp Gln Glu Thr Thr Thr Gln Gly Pro Gly Val Leu  
 15 20 25  
 CTT CCC CTG CCC AAG GGG GCC TGC ACA GGC TGG ATG GCG GGC ATC CCA 204  
 Leu Pro Leu Pro Lys Gly Ala Cys Thr Gly Trp Met Ala Gly Ile Pro  
 30 35 40  
 GGG CAT CCG GGC CAT AAT GGG GCC CCA GGC CGT GAT GGC AGA GAT GGC 252  
 Gly His Pro Gly His Asn Gly Ala Pro Gly Arg Asp Gly Arg Asp Gly  
 45 50 55 60  
 ACC CCT GGT GAG AAG GGT GAG AAA GGA GAT CCA GGT CTT ATT GGT CCT 300  
 Thr Pro Gly Glu Lys Gly Glu Lys Gly Asp Pro Gly Leu Ile Gly Pro  
 65 70 75  
 AAG GGA GAC ATC GGT GAA ACC GGA GTA CCC GGG GCT GAA GGT CCC CGA 348  
 Lys Gly Asp Ile Gly Glu Thr Gly Val Pro Gly Ala Glu Gly Pro Arg  
 80 85 90

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GGC TTT CCG GGA ATC CAA GGC AGG AAA GGA GAA CCT GGA GAA GGT GCC Gly Phe Pro Gly Ile Gln Gly Arg Lys Gly Glu Pro Gly Glu Gly Ala 95 100 105	396
TAT GTA TAC CGC TCA GCA TTC AGT GTG GGA TTG GAG ACT TAC GTT ACT Tyr Val Tyr Arg Ser Ala Phe Ser Val Gly Leu Glu Thr Tyr Val Thr 110 115 120	444
ATC CCC AAC ATG CCC ATT CGC TTT ACC AAG ATC TTC TAC AAT CAG CAA Ile Pro Asn Met Pro Ile Arg Phe Thr Lys Ile Phe Tyr Asn Gln Gln 125 130 135 140	492
AAC CAC TAT GAT GGC TCC ACT GGT AAA TTC CAC TGC AAC ATT CCT GGG Asn His Tyr Asp Gly Ser Thr Gly Lys Phe His Cys Asn Ile Pro Gly 145 150 155	540
CTG TAC TAC TTT GCC TAC CAC ATC ACA GTC TAT ATG AAG GAT GTG AAG Leu Tyr Tyr Phe Ala Tyr His Ile Thr Val Tyr Met Lys Asp Val Lys 160 165 170	588
GTC AGC CTC TTC AAG AAG GAC AAG GCT ATG CTC TTC ACC TAT GAT CAG Val Ser Leu Phe Lys Lys Asp Lys Ala Met Leu Phe Thr Tyr Asp Gln 175 180 185	636
TAC CAG GAA AAT AAT GTG GAC CAG GCC TCC GGC TCT GTG CTC CTG CAT Tyr Gln Glu Asn Asn Val Asp Gln Ala Ser Gly Ser Val Leu Leu His 190 195 200	684
CTG GAG GTG GGC GAC CAA GTC TGG CTC CAG GTG TAT GGG GAA GGA GAG Leu Glu Val Gly Asp Gln Val Trp Leu Gln Val Tyr Gly Glu Gly Glu 205 210 215 220	732
CGT AAT GGA CTC TAT GCT GAT AAT GAC AAT GAC TCC ACC TTC ACA GGC Arg Asn Gly Leu Tyr Ala Asp Asn Asp Asn Asp Ser Thr Phe Thr Gly 225 230 235	780
TTT CTT CTC TAC CAT GAC ACC AAC TGATCACCAC TAACTCAGAG CCTCCTCCAG Phe Leu Leu Tyr His Asp Thr Asn 240	834
GCCAAACAGC CCCAAAGTCA ATTAAAGGCT TTCAGTACGG TTAGGAAGTT GATTATTATT	894
TAGTTGGAGG CCTTTAGATA TTATTCATTC ATTACTCAT TCATTTATTC ATTCATTCAT	954
CAAGTAACTT TAAAAAATC ATATGCTATG TTCCAGTCC TGGGGAGCTT CACAAACATG	1014
ACCAGATAAC TGACTAGAAA GAAGTAGTTG ACAGTGCTAT TTCGTGCCCA CTGTCTCTCC	1074
TGATGCTCAT ATCAATCCTA TAAGGCACAG GGAACAAGCA TTCTCCTGTT TTTACAGATT	1134
GTATCCTGAG GCTGAGAGAG TTAAGTGAAT GTCTAAGGTC ACACAGTATT AAGTGACAGT	1194
GCTAGAAATC AAACCCAGAG CTGTGGACTT TGTTCACTAG ACTGTGCCCC TTTTATAGAG	1254
GGTACATGTT CTCTTTGGAG TGTGGTAGG TGTCTGTTTC CCACCTCACC TGAGAGCCA	1313



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## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 244 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Leu Leu Leu Gly Ala Val Leu Leu Leu Leu Ala Leu Pro Gly His  
 1 5 10 15  
 Asp Gln Glu Thr Thr Thr Gln Gly Pro Gly Val Leu Leu Pro Leu Pro  
 20 25 30  
 Lys Gly Ala Cys Thr Gly Trp Met Ala Gly Ile Pro Gly His Pro Gly  
 35 40 45  
 His Asn Gly Ala Pro Gly Arg Asp Gly Arg Asp Gly Thr Pro Gly Glu  
 50 55 60  
 Lys Gly Glu Lys Gly Asp Pro Gly Leu Ile Gly Pro Lys Gly Asp Ile  
 65 70 75 80  
 Gly Glu Thr Gly Val Pro Gly Ala Glu Gly Pro Arg Gly Phe Pro Gly  
 85 90 95  
 Ile Gln Gly Arg Lys Gly Glu Pro Gly Glu Gly Ala Tyr Val Tyr Arg  
 100 105 110  
 Ser Ala Phe Ser Val Gly Leu Glu Thr Tyr Val Thr Ile Pro Asn Met  
 115 120 125  
 Pro Ile Arg Phe Thr Lys Ile Phe Tyr Asn Gln Gln Asn His Tyr Asp  
 130 135 140  
 Gly Ser Thr Gly Lys Phe His Cys Asn Ile Pro Gly Leu Tyr Tyr Phe  
 145 150 155 160  
 Ala Tyr His Ile Thr Val Tyr Met Lys Asp Val Lys Val Ser Leu Phe  
 165 170 175  
 Lys Lys Asp Lys Ala Met Leu Phe Thr Tyr Asp Gln Tyr Gln Glu Asn  
 180 185 190  
 Asn Val Asp Gln Ala Ser Gly Ser Val Leu Leu His Leu Glu Val Gly  
 195 200 205  
 Asp Gln Val Trp Leu Gln Val Tyr Gly Glu Gly Glu Arg Asn Gly Leu  
 210 215 220  
 Tyr Ala Asp Asn Asp Asn Asp Ser Thr Phe Thr Gly Phe Leu Leu Tyr  
 225 230 235 240  
 His Asp Thr Asn

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## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 15 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Glu Asp Asp Val Thr Thr Thr Glu Glu Leu Ala Pro Ala Leu Val  
1                  5                  10                  15

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CLAIMS

We claim:

1. Isolated or recombinantly produced DNA encoding mammalian adipocyte complement related protein.
2. The DNA of Claim 1 wherein the DNA is selected from the group consisting of: DNA encoding human adipocyte complement related protein and DNA encoding rodent adipocyte complement related protein.
3. The DNA of Claim 2 wherein the nucleotide sequence is selected from the group consisting of: SEQ ID NO:1, complements of SEQ ID NO:1, SEQ ID NO:6, complements of SEQ ID NO:6 and portions thereof.
4. DNA comprising a nucleotide sequence selected from the group consisting of: SEQ ID No: 1, a complement of SEQ ID NO:1, SEQ ID NO: 6, a complement of SEQ ID NO:6 and portions thereof.
5. DNA encoding mammalian adipocyte complement related protein, wherein the protein comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:7, and portions thereof.
6. DNA which hybridizes to DNA selected from the group consisting of: SEQ ID No:1, a complement of SEQ ID NO:1, SEQ ID NO:6, a complement of SEQ ID NO:6 and DNA which hybridizes to portions thereof.
7. RNA transcribed from DNA selected from the group consisting of: SEQ ID NO:1, a complement of SEQ ID

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NO:1, SEQ ID NO:6, a complement of SEQ ID NO:6 and portions thereof.

8. An expression vector comprising DNA selected from the group consisting of: SEQ ID No: 1, SEQ ID NO: 6 and portions thereof.
9. Isolated or recombinantly produced mammalian adipocyte complement related protein.
10. The protein of Claim 9 wherein the protein is selected from the group consisting of: human adipocyte complement related protein and mouse adipocyte complement related protein.
11. The protein of Claim 10 wherein the amino acid sequence of the human adipocyte complement related protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID No: 7 and functional portions thereof.
12. A protein comprising an amino acid sequence selected from the group consisting of: SEQ ID No:2, SEQ ID NO: 7 and functional portions thereof.
13. An inhibitor of mammalian adipocyte complement related protein.
14. An inhibitor of Claim 13 wherein the inhibitor is an antibody which binds adipocyte complement related protein or a functional portion of adipocyte complement related protein.
15. The antibody of Claim 14 which binds a protein wherein the amino acid sequence of the protein is selected

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from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 7 and functional portions thereof.

16. The antibody of Claim 15 wherein the antibody is selected from the group consisting of: monoclonal antibodies, chimeric antibodies and humanized antibodies.
17. A method of detecting mammalian adipocyte complement related protein in a sample of cells obtained from an individual, comprising the steps of:
- a) treating the sample to render nucleic acids in the sample available for hybridization to a nucleic acid probe, thereby producing a treated sample;
  - b) combining the treated sample with a nucleic acid probe comprising all or a functional portion of the nucleotide sequence of mammalian adipocyte complement related protein, under conditions appropriate for hybridization of complementary nucleic acids; and
  - c) detecting hybridization of the treated sample with the labeled nucleic acid probe, wherein hybridization indicates the presence of mammalian adipocyte complement related protein in the sample.
18. A method of Claim 17 wherein the nucleic acid probe comprises DNA selected from the group consisting of: SEQ ID No: 1, SEQ ID NO: 6, and portions thereof.
19. A method of Claim 17 wherein the sample is human blood.

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20. A method of detecting mammalian adipocyte complement related protein in a sample obtained from an individual, comprising the steps of:
- a) combining the sample with an antibody which binds adipocyte complement related protein or a functional portion of adipocyte complement related protein; and
  - b) detecting binding of the antibody to a component of the sample,
- wherein binding of the antibody to a component of the sample indicates the presence of mammalian adipocyte complement related protein in the sample.
21. A method of Claim 20 wherein the antibody binds a protein comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 7 and portions thereof.
22. A method of altering the energy balance in a mammal, comprising administering to the mammal an agent which interacts with the adipocyte complement related protein.
23. A method of detecting adipocytes in a sample of cells obtained from an individual, comprising the steps of:
- a) treating the sample to render nucleic acids in cells in the sample available for hybridization to a nucleic acid probe, thereby producing a treated sample;
  - b) combining the treated sample with a labelled nucleic acid probe having all or a portion of the nucleotide sequence of mammalian adipocyte complement related protein, under conditions appropriate for hybridization of complementary nucleic acids; and

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- c) detecting hybridization of the treated sample with the labeled nucleic acid probe, wherein hybridization indicates the presence of adipocytes in the sample.
- 5 24. A method of Claim 23 wherein the nucleic acid probe comprises DNA selected from the group consisting of: SEQ ID No: 1, SEQ ID NO: 6, and portions thereof.
25. A method of Claim 23 wherein the sample is human blood.
- 10 26. The protein of Claim 9 which is secreted by adipocytes, the secretion is enhanced by insulin.
27. A method of modulating insulin production in a mammal comprising administering adipocyte complement related protein to the mammal.
- 15 28. The method of Claim 27 wherein adipocyte complement related protein is administered by means of introducing into the mammal cells which contain DNA encoding adipocyte complement related protein which is expressed and secreted.
- 20 29. Use of adipocyte complement related protein to modulate insulin production in a mammal.

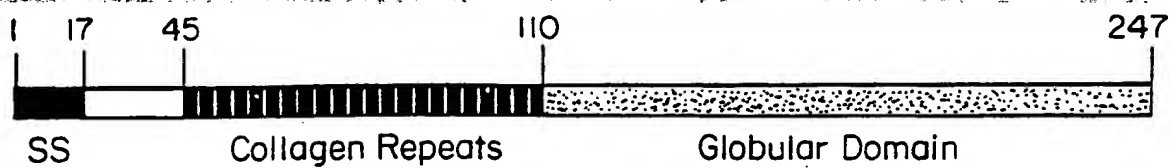
1/6

1  
 CTC TAA AGA TTG TCA GTG GAT CTG ACG ACA CCA AAA GGG CTC AGG ATG CTA CTG TTG CAA  
 M L L L G  
 61  
 GCT CTC CTG TTC CTC TTA ATC CTG CCC AGT CAT GCC GAA GAT GAC GTT ACT ACA ACT GAA  
 A L L F L L I L P S H A E D D V T T T E  
 121  
 GAG CTA GCT CCT GCT TTG GTC CCT CCA CCC AAG GGA ACT TGT GCA GGT TGG ATG GCA GGC  
 E L A P A L V P P P K G T C A G W M A G  
 181  
 ATC CCA GGA CAT CCT GGC CAC AAT GGC ACA CCA GGC CGT GAT GGC AGA GAT GGC ACT CCT  
 I P G H P G H N G T P G R D G R D G T P  
 241  
 GGA GAG AAG GGA GAG AAA GGA GAT GCA GGT CTT CTT GGT CCT AAG GGT GAG ACA GGA GAT  
 G E K G E K G D A G L L G P K G E T G D  
 301  
 GTT GGA ATG ACA GGA GCT GAA GGG CCA CGG GGC TTC CCC GGA ACC CCT GGC AGG AAA GGA  
 V G M T G A E G P R G F P G T P G R K G  
 361  
 GAG CCT GGA GAA GCC GCT TAT ATG TAT CGC TCA GCG TTC AGT GTG GGG CTG GAG ACC CGC  
 E P G E A A Y M Y R S A F S V G L E T R  
 421  
 GTC ACT GTT CCC AAT GTA CCC ATT CGC TTT ACT AAG ATC TTC TAC AAC CAA CAG AAT CAT  
 V T V P N V P I R F T K I F Y N Q Q N H  
 481  
 TAT GAC GGC AGC ACT GGC AAG TTC TAC TGC AAC ATT CCG GGA CTC TAC TAC TTC TCT TAC  
 Y D G S T G K F Y C N I P G L Y Y F S Y  
 541  
 CAC ATC ACG GTG TAC ATG AAA GAT GTG AAG GTG AGC CTC TTC AAG AAG GAC AAG GCC GTT  
 H I T V Y M K D V K V S L F K K D K A V  
 601  
 CTC TTC ACC TAC GAC CAG TAT CAG GAA AAG AAT GTG GAC CAG GCC TCT GGC TCT GTG CTC  
 L F T Y D Q Y Q E K N V D Q A S G S V L  
 661  
 CTC CAT CTG GAG GTG GGA GAC CAA GTC TGG CTC CAG GTG TAT GGG GAT GGG GAC CAC AAT  
 L H L E V G D Q V W L Q V Y G D G D H N  
 721  
 GGA CTC TAT GCA GAT AAC GTC AAC GAC TCT ACA TTT ACT GGC TTT CTT CTC TAC CAT GAT  
 G L Y A D N V N D S T F T G F L L Y H D  
 781  
 ACC AAC TGA CTG CAA CTA CCC ATA GCC CAT ACA CCA GGA GAA TCA TGG AAC AGT CGA CAC  
 T N \*  
 841  
 ACT TTC AGC TTA GTT TGA GAG ATT GAT TTT ATT GCT TAG TTT GAG AGT CCT GAG TAT TAT  
 901  
 CCA CAC GTG TAC TCA CTT GTT CAT TAA ACG ACT TTA TAA AAA ATA ATT TGT GTT CCT AGT  
 961  
 CCA GAA AAA AAG GCA CTC CCT GGT CTC CAC GAC TCT TAC ATG GTA GCA ATA ACA GAA TGA  
 1021  
 AAA TCA CAT TTG GTA TGG GGG CTT CAC AAT ATT CGC ATG ACT GTC TGG AAG TAG ACC ATG  
 1081  
 CTA TTT TTC TGC TCA CTG TAC ACA AAT ATT GTT CAC ATA AAC CCT ATA ATG TAA ATA TGA  
 1141  
 AAT ACA GTG ATT ACT CTT CTC ACA GGC TGA GTG TAT GAA TGT CTA AAG ACC CAT AAG TAT  
 1201  
 TAA AGT GGT AGG GAT AAA TTG GAA AAA AAA AAA AAA AAA AAG AAA AAC TTT AGA GCA CAC  
 1261  
 TGG CGG CCG TTA CTA G

FIG. 1

SUBSTITUTE SHEET (RULE 26)





**FIG. 2**



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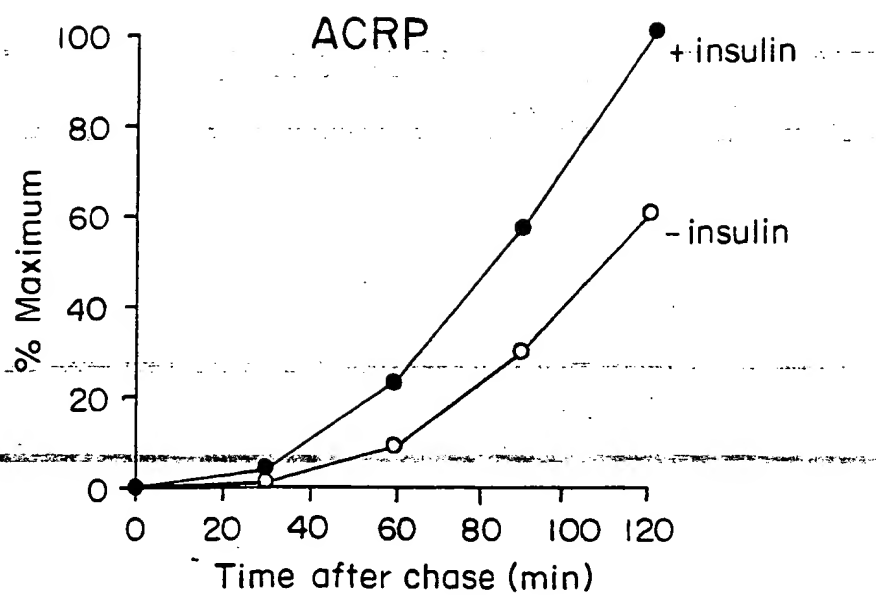


FIG. 4A

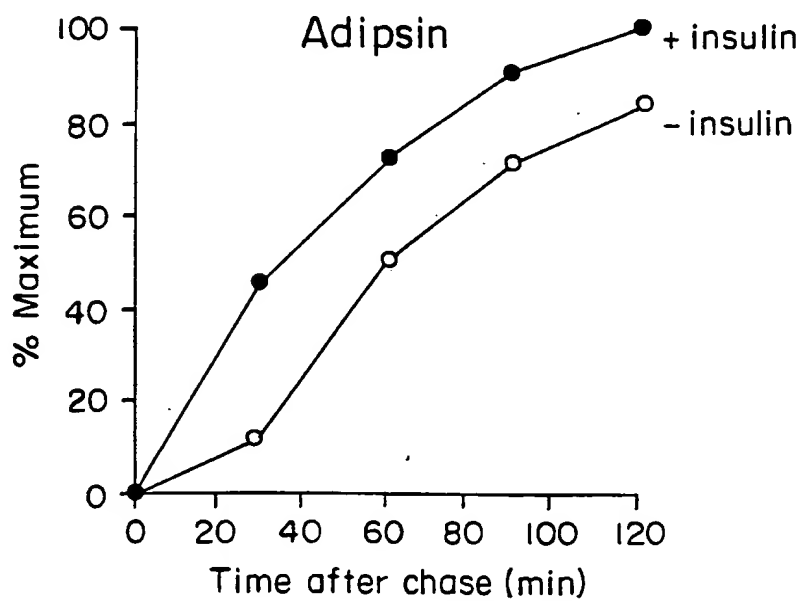


FIG. 4B

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AGG TCG ACG GTA TCG ATA AGC TTG ATA TCG AAT TCC GGC TGC GGT TCT GAT TCC ATA CCA  
 GAG GGG CTC AGG ATG CTG TTG CTG GGA GCT GTT CTA CTG CTA TTA GCT CTG CCC GGT CAT  
 M L L L G A V L L L L A L P G H  
 GAC CAG GAA ACC ACG ACT CAA GGG CCC GGA GTC CTG CTT CCC CTG CCC AAG GGG GCC TGC  
 D Q E T T T Q G P G V L L P L P K G A C  
 ACA GGC TGG ATG GCG GGC ATC CCA GGG CAT CCG GGC CAT AAT GGG GCC CCA GGC CGT GAT  
 T G W M A G I P G H P G H N G A P G R D  
 GGC AGA GAT GGC ACC CCT GGT GAG AAG GGT GAG AAA GGA GAT CCA GGT CTT ATT GGT CCT  
 G R D G T P G E K G E K G D P G L I G P  
 AAG GGA GAC ATC GGT GAA ACC GGA GTA CCC GGG GCT GAA GGT CCC CGA GGC TTT CCG GGA  
 K G D I G E T G V P G A E G P R G F P G  
 ATC CAA GGC AGG AAA GGA GAA CCT GGA GAA GGT GCC TAT GTA TAC CGC TCA GCA TTC AGT  
 I Q G R K G E P G E G A Y V Y R S A F S  
 GTG GGA TTG GAG ACT TAC GTT ACT ATC CCC AAC ATG CCC ATT CGC TTT ACC AAG ATC TTC  
 V G L E T Y V T I P N M P I R F T K I F  
 TAC AAT CAG CAA AAC CAC TAT GAT GGC TCC ACT GGT AAA TTC CAC TGC AAC ATT CCT GGG  
 Y N Q Q N H Y D G S T G K F H C N I P G  
 CTG TAC TAC TTT GCC TAC CAC ATC ACA GTC TAT ATG AAG GAT GTG AAG GTC AGC CTC TTC  
 L Y Y F A Y H I T V Y M K D V K V S L F  
 AAG AAG GAC AAG GCT ATG CTC TTC ACC TAT GAT CAG TAC CAG GAA AAT AAT GTG GAC CAG  
 K K D K A M L F T Y D Q Y Q E N N V D Q  
 GCC TCC GGC TCT GTG CTC CTG CAT CTG GAG GTG GGC GAC CAA GTC TGG CTC CAG GTG TAT  
 A S G S V L L H L E V G D Q V W L Q V Y  
 GGG GAA GGA GAG CGT AAT GGA CTC TAT GCT GAT AAT GAC AAT GAC TCC ACC TTC ACA GGC  
 G E G E R N G L Y A D N D N D S T F T G  
 TTT CTT CTC TAC CAT GAC ACC AAC TGA TCA CCA CTA ACT CAG AGC CTC CTC CAG GCC AAA  
 F L L Y H D T N \*  
 CAG CCC CAA AGT CAA TTA AAG GCT TTC AGT ACG GTT AGG AAG TTG ATT ATT ATT TAG TTG  
 GAG GCC TTT AGA TAT TAT TCA TTC ATT TAC TCA TTC ATT TAT TCA TTC ATT CAT CAA GTA  
 ACT TTA AAA AAA TCA TAT GCT ATG TTC CCA GTC CTG GGG AGC TTC ACA AAC ATG ACC AGA  
 TAA CTG ACT AGA AAG AAG TAG TTG ACA GTG CTA TTT CGT GCC CAC TGT CTC TCC TGA TGC  
 TCA TAT CAA TCC TAT AAG GCA CAG GGA ACA AGC ATT CTC CTG TTT TTA CAG ATT GTA TCC  
 TGA GGC TGA GAG AGT TAA GTG AAT GTC TAA GGT CAC ACA GTA TTA AGT GAC AGT GCT AGA  
 AAT CAA ACC CAG AGC TGT GGA CTT TGT TCA CTA GAC TGT GCC CCT TTT ATA GAG GGT ACA  
 TGT TCT CTT TGG AGT GTT GGT AGG TGT CTG TTT CCC ACC TCA CCT GAG AGC CA

FIG. 5



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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C12N 15/12, C07K 14/47, C12Q 1/70,</b> <b>C07K 16/18, G01N 33/50, A61K 38/17</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 96/39429</b> <b>(43) International Publication Date:</b> 12 December 1996 (12.12.96)
<b>(21) International Application Number:</b> PCT/US96/08629 <b>(22) International Filing Date:</b> 4 June 1996 (04.06.96) <b>(30) Priority Data:</b> 08/463,911 5 June 1995 (05.06.95) <b>US</b> <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> <b>US</b> 463,911 (CIP) <b>Filed on</b> 5 June 1995 (05.06.95) <b>(71) Applicant (for all designated States except US):</b> WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH [US/US]; Nine Cambridge Center, Cambridge, MA 02142 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SCHERER, Philipp, E. [CH/US]; 316 Lexington Street, Watertown, MA 02172 (US). LODISH, Harvey, F. [US/US]; 195 Fisher Avenue, Brookline, MA 02146 (US). <b>(74) Agents:</b> GRANAHAH, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).	<b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i> <b>(88) Date of publication of the international search report:</b> 30 January 1997 (30.01.97)	
<b>(54) Title:</b> SERUM PROTEIN PRODUCED EXCLUSIVELY IN ADIPOCYTES		
<b>(57) Abstract</b> <p>The present invention relates to DNA encoding Acrp30, of vertebrate (e.g. mammalian) origin, and particularly of human and rodent origin. The present invention further relates to isolated, recombinantly produced or synthetic DNA which hybridizes to the nucleotide sequences described herein and RNA transcribed from the nucleotides sequence described herein. In addition, the invention relates to expression vectors comprising DNA encoding Acrp30, which is expressed when the vector is present in an appropriate host cell. The invention further relates to isolated, recombinantly produced or synthetic mammalian Acrp30 of vertebrate (e.g., mammalian) origin, and particularly of human and rodent origin. Also encompassed by the present invention is an inhibitor or enhancer of Acrp30. The present invention further relates to a method of identifying inhibitors or enhancers of Acrp30. Isolation of Acrp30 makes it possible to detect Acrp30 or adipocytes in a sample (e.g., test sample). In addition, the present invention relates to a method of regulating the energy balance (e.g., nutritional status) of a mammal by administering to the mammal an inhibitor or enhancer of the Acrp30. The present invention further relates to a method of modulating insulin production in a mammal comprising administering Acrp30 to the mammal.</p>		

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## INTERNATIONAL SEARCH REPORT

International Application No.

PC1/US 96/08629

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12Q1/70 C07K16/18 G01N33/50  
A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBL, accession no S23297, sequence reference S22243, 07-10-1994, chicken mRNA complement C1q carboxy-terminal homology, XP002020243 see the whole document ---	1
P,X	EMBL, accession number U37222, sequence reference g1051267, 7-11-1995, Mus musculus 30 kDa adipocyte complement- XP002020244 related protein Acrp30 mRNA see the whole document --- -/--	1-13, 17-19, 23-26

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

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## INTERNATIONAL SEARCH REPORT

International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY (MICROFILMS), vol. 270, no. 45, 10 November 1995, MD US, pages 26746-26749, XP000612012 SCHERER, P.E. ET AL.: "A novel serum protein similar to Clq, produced exclusively in adipocytes" see the whole document ---	9-12
P,X	EMBL, accession no D45371, sequence reference g871886, 28-06-1995, Human apM1 mRNA for GS3109 XP002020245 see the whole document -----	1-13, 17-19, 23-26
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 221, 16 April 1996, ORLANDO, FL US, pages 286-289, XP000612064 MAEDA, R. ET AL.: "cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1)" see the whole document -----	1-12,17, 18,20

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/08629

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
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Remark: Although claim(s) 22, 27-29  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
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**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

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